Attorney Docket No: SALK 3130-1S
(088802-9706)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Downes, M. et al.

Title: NON-STEROIDAL FARNESOID X RECEPTOR MODULATORS AND METHODS FOR THE USE THEREOF

Appl. No.: 10/535,043

Filing Date: May 13, 2005

Examiner: Paul E. Zarek

Art Unit: 1617

Conf. No: 2033

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

We, Michael Downes and Ronald M. Evans, being duly warned, hereby declare and say that:

1. I, Michael Downes, am a citizen of Australia, residing in San Diego, CA. I am currently employed by the Salk Institute for Biological Studies as a post-doctoral fellow in the Gene Expression Laboratory (in the laboratory of Dr. Ronald M. Evans).

2. I, Ronald M. Evans, am a citizen of the United States, residing in La Jolla, CA. I am currently employed by the Howard Hughes Medical Institute at the Salk Institute for Biological Studies as a Professor in the Gene Expression Laboratory.

3. We are the sole co-inventors of the invention disclosed and claimed in the above-referenced application.

4. We are familiar with the PCT publication entitled "NON-STEROIDAL FXR AGONISTS", published as international publication number WO 2004/046162, on 3 June 2004, based on two priority filings, the earliest of which was 14 November 2002. We understand that this publication discloses subject matter relating to the present application, and that this publication has been cited as prior art against the present application by the Examiner.

5. We conceived of the invention claimed in the above-referenced application prior to the earliest priority date to which the '162 publication may be entitled, as reflected by the attached "INVENTION DISCLOSURE FORM", which bears a date prior to 14 November 2002 (the attached is a true copy of the original disclosure form, with only the dates thereon having been redacted).

6. We individually and collectively further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

6/23/09
Date

M. Downes
Michael Downes

6/23/09
Date

Ronald M. Evans
Ronald M. Evans

INVENTION DISCLOSURE FORM

Title: Development of non-steroidal Farnesoid X Receptor (FXR) agonists

Description of Invention: Using cell based HTS technology developed in the Evans laboratory we screened a 10 000-membered, diversity-orientated library of benzopyran containing small molecules constructed by the Nicolaou lab (1,2) for transcriptional activation of the FXR (3). (Details pertaining to the development and implementation of the cell-based assay are described in Appendix I). The initial primary screen of the library revealed 25 compounds. In collaboration with the Nicolaou lab focused compound libraries were synthesized around these hits and re-assayed for FXR activation. Ten rounds of this process produced four classes of FXR activators. These compounds are termed 1) fexaramate (EC_{50} 127nM); 2) fexarene (EC_{50} 36nM); 3) fexaramine (EC_{50} 25nM); 4) fexarine (EC_{50} 38nM); 5) fexachloramide (EC_{50} 188nM) (see figure 1A for structures). These compounds were then re-tested in a diverse array of both in vivo and an in vitro FRET based assays (figure1B and 2). In addition these compounds have been tested for cross reactivity with other nuclear hormone receptors and display specificity for FXR activation (figure 3). These compounds also activate the natural promoters of known FXR target genes like IBABP, PLTP and the MRP-2 gene in transient transfection and endogenous cell based experiments Figure 4.

Details of Problem Which is Solved by Invention:
FXR is a bile acid receptor helping to control cholestrol metabolism in the liver and lipid absorption in the gut (4,5). The discovery of these specific chemical tools enables us to manipulate FXR function in the body. Natural ligands such as BAs possess multiple receptor independent physiologic activities. BAs also activate other NRs such as LXR, PXR and VDR as well as c-Jun N-terminal kinase JNK. Our compounds are high affinity selective compounds for FXR activation and thus provide a unique tool for altering FXR function. In vivo these compounds could have utility to influence Bile Acid (BA) production in the liver, BA flow to the gall bladder and BA release and absorption in the intestine. This could impact on biliary cholestasis, gallstones and BA absorption and metabolism. In addition bile acids, need special carrier proteins such as IBAT (Intestinal bile acid transporter) to get to into the cell where as these ligands freely permeate into the cell and are not dependant on transporters.

Probable Utilization of Invention:
The probably use of this invention is in the disease state of biliary cholestasis and to manipulate the bile acid metabolism in the body.

Description of Previously Known or Published Materials: *(Give literature references, or patent numbers, or attach illustrations.)*

1. Nicolaou KC et al., J. Am. Chem. Soc 122, 9954-9967.
2. Nicolaou KC et al., J. Am. Chem. Soc 122, 9968-9976.
3. Forman BM et al., Cell J 2;81(5):687-93.
3. Parks DJ et al., Science 284(5418), 1365-1368.
4. Maskishima M et al., Science 284(5418) 1362-1365.

Advantages Over What was Previously Known:
Bile acids and synthetic resins, which absorb bile acids, are currently used to treat human disease. These compounds provide a potential pharmacological approach to the treatment of bile related disease.

Invention Record

Conception: Date:

First Written Description: Date: In _____

First Disclosure to Others: Date: To _____
Written Oral

Funding Information

	Agency	Grant Number
1)		
2)		
3)		

Inventors' Full Names and Addresses *

	Last	First	Middle	Home P.O. Address	Citizenship
1)	Downes	Michael	R.	3440 Lebon Dr. #4210 San Diego, CA 92122	Australia
2)	Evans	Ronald	M.	1471 Cottontail Ln. La Jolla, CA 92037	USA

Inventors' Signatures

Date: _____ _____

Date: _____ _____

Date: _____ _____

Invention Disclosure Read and Understood By **

Date: _____ _____

Date: _____ _____

* *More than one inventor may be named, but each inventor must have participated in the conception of the invention.*

** *Should be persons other than inventors.*

Figure 1A

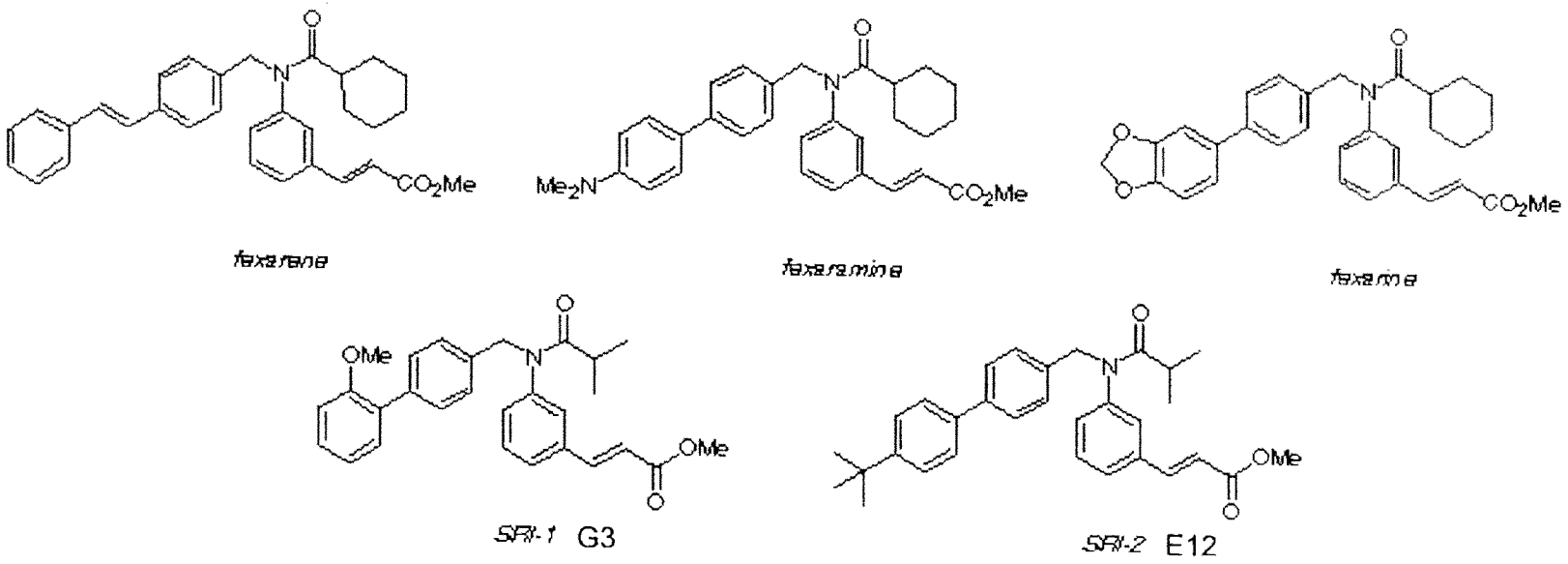


Figure 1B

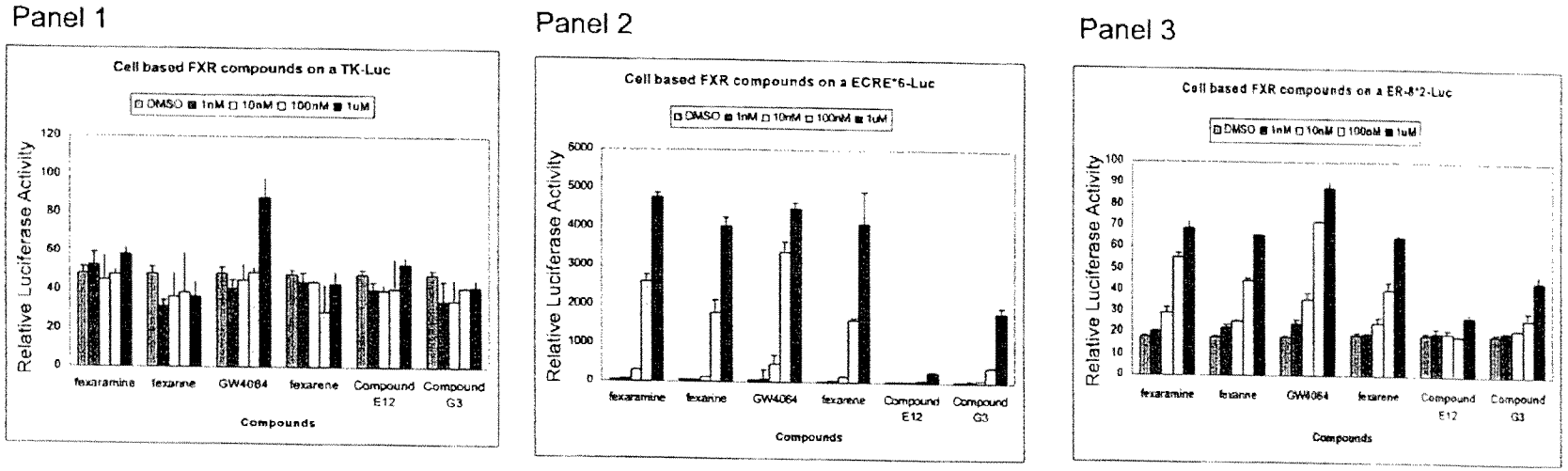
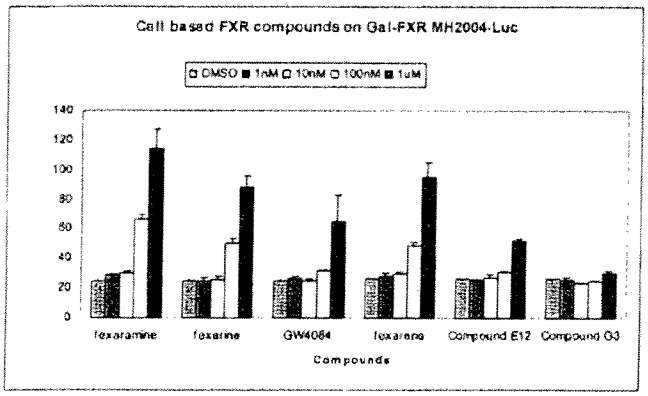
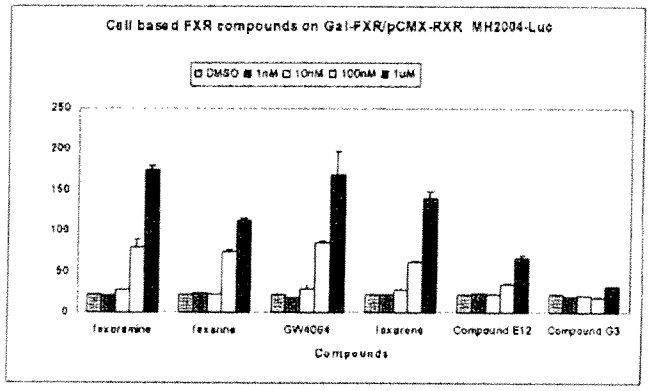
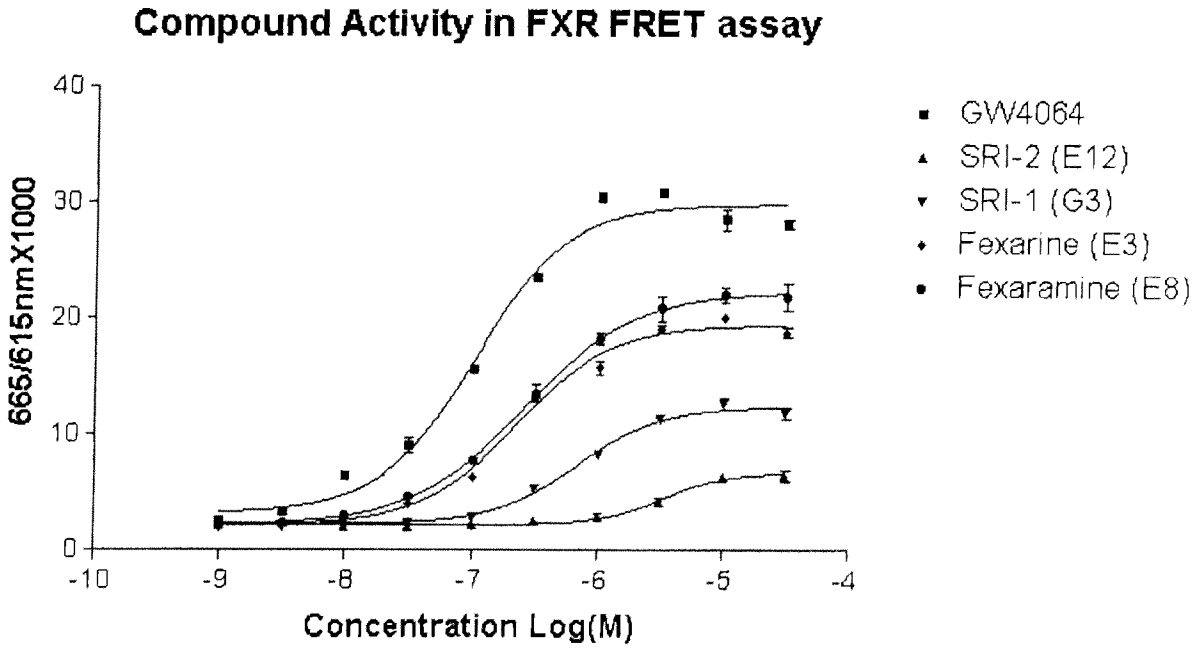


Figure 2 A





B.



GW4064	SRI-1 (G3)	SRI-2 (E12)	Fexarine	Fexaramine
1.0330e-007	3.3720e-006	6.8730e-007	2.2280e-007	2.5520e-007

Figure 3

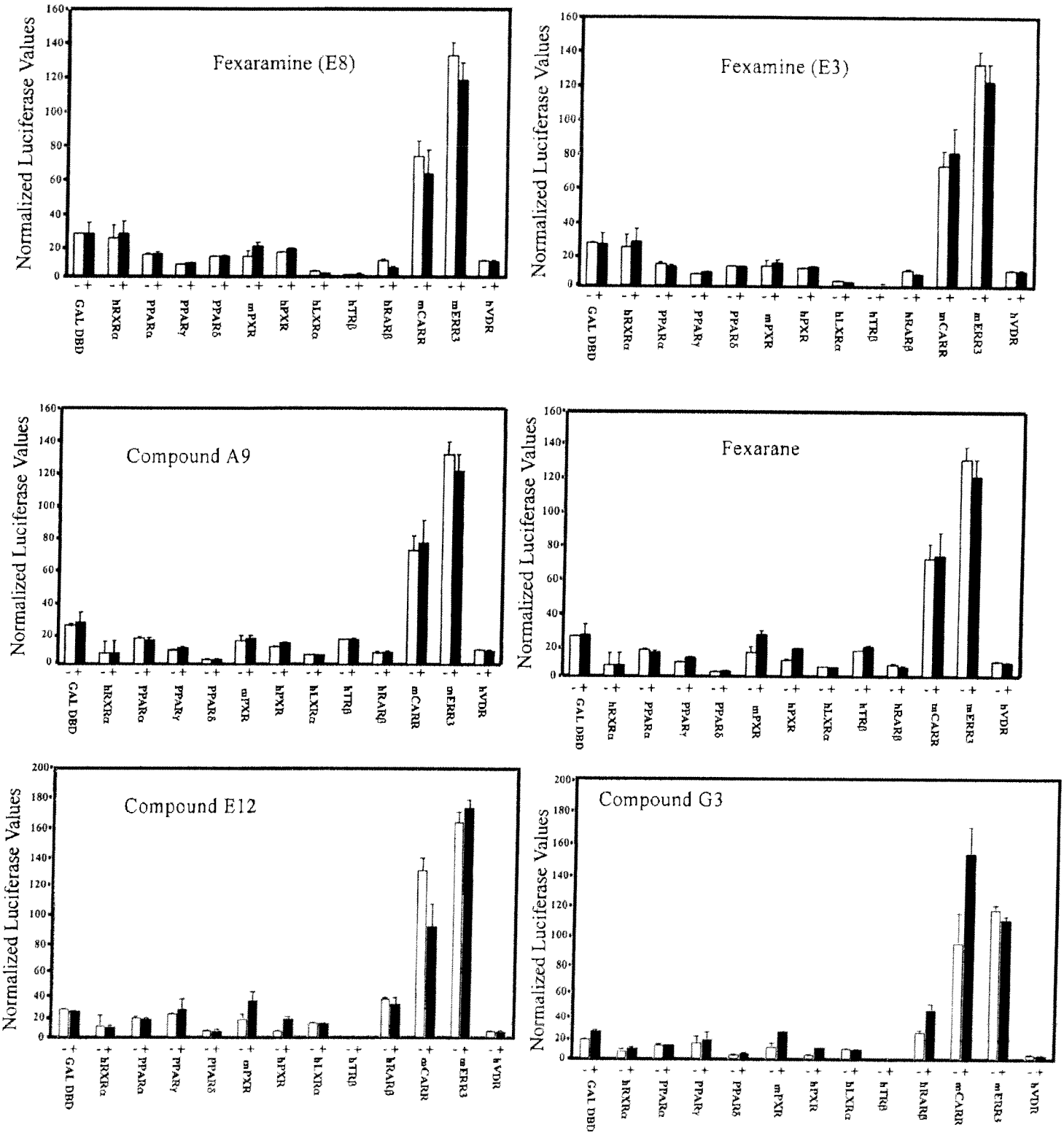
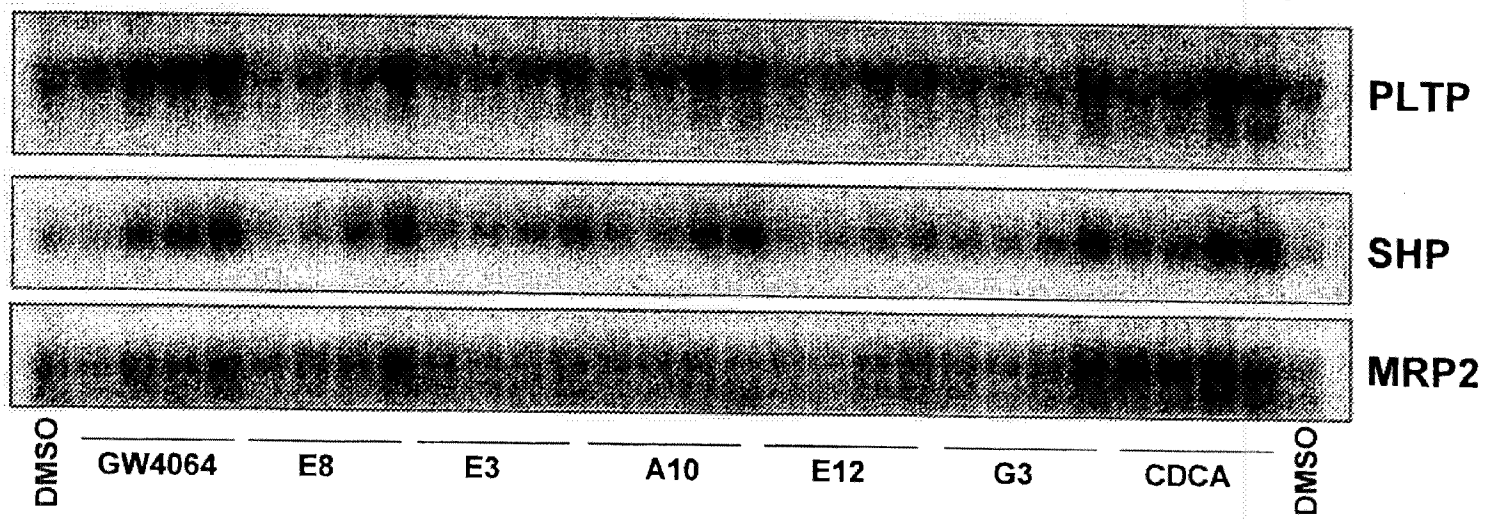
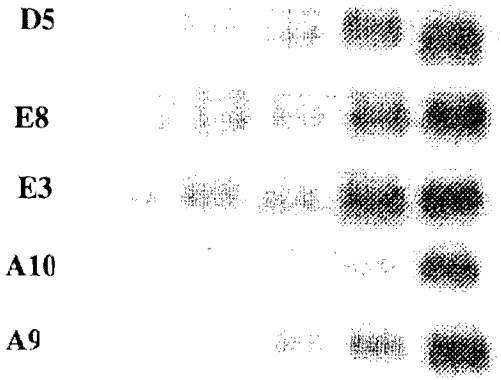

Fexaramine (E8)
Fexamine (E3)
Compound A9
Fexarane
Compound E12
Compound G3
Normalized Luciferase Values
GAL DBD
hRXRα
PPARα
PPARγ
PPARδ
mPXR
hPXR
hLXRα
hTRβ
hRARβ
mCARR
mERR3
hVDR

Figure 4

HepG2 cell s induction of target genes by novel compounds



HT29 FXR compounds



HT29-colon cell line
HepG2-Liver cell line

Appendix I

Development of cell based assay and implementation of screen:
We describe the development and employment of a high throughput nuclear hormone receptor screening platform that has resulted in the discovery of a novel chemical tools that function as synthetic ligands for the orphan nuclear receptor farnesoid X receptor. The screen consists of both in vivo and invitro based assays developed in a high throughput (HTS) 384 well format. The assays exploit the fact that the direct interaction of receptor LBDs with nuclear receptor co-regulators and their derivative peptides is highly dependent on the presence of a specific activating ligand (Heery *et al.*, 1997). A central component of the *in vivo* technology platform is the conversion of standard manual assays employing millions of cells per point (i.e., one plate) to a miniaturized format e.g., 96-wells (5,000 cells per well) or 384 wells (*1250* cells per well) which conforms to robotic platforms.

In vivo assay:
The feasibility of creating high throughput screens (HTS) for ORs was explored using FXR as candidate OR with a known activator, chenodeoxycholic acid (CDCA) as a ligand. The screen is based on the co-transfection of a full-length receptor with the reporter vector containing a natural hormone response element under a minimal eukaryotic promoter. Our results (Figure 1) demonstrate that we can successfully screen in a dose dependent manner for potential activating chemical ligands using a full length FXR on a natural response element. These results validate the robustness of the assay for FXR, in 384-well plates. Using this 384 -well format we applyed the HTS approach to FXR as a candidate OR. For this test screen we used a 10 000 membered library constructed around the privileged 2,2-dimethylbenopyran scaffold from the Nicolaou lab (Nicolaou *et al.*, 2000). This library comprises approximately 10 000 distinct compounds with structures and sizes similar to natural products such as phyto-estrogens, flavanoids, coumarins and long chain fatty acids. A central question in the feasibility studies is whether this library is suitable for screening for NR ligands. Samples of this library were first reformatted into a 384-well format and then subjected to the FXR cell-based assays as described above and assessed for FXR-mediated transcriptional activity. Cells were exposed to approximately 10μM of sample for 18 hrs prior to washing and luciferase analysis (results shown in figure 2).

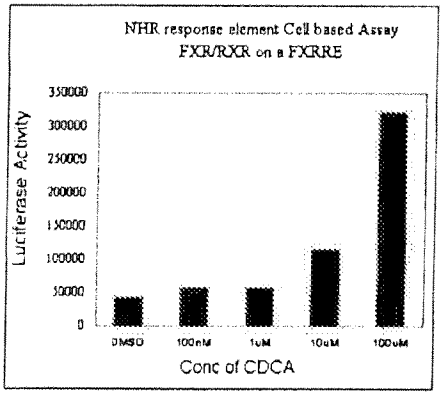


Figure 1. FXR efficacy on a 384 well plate.

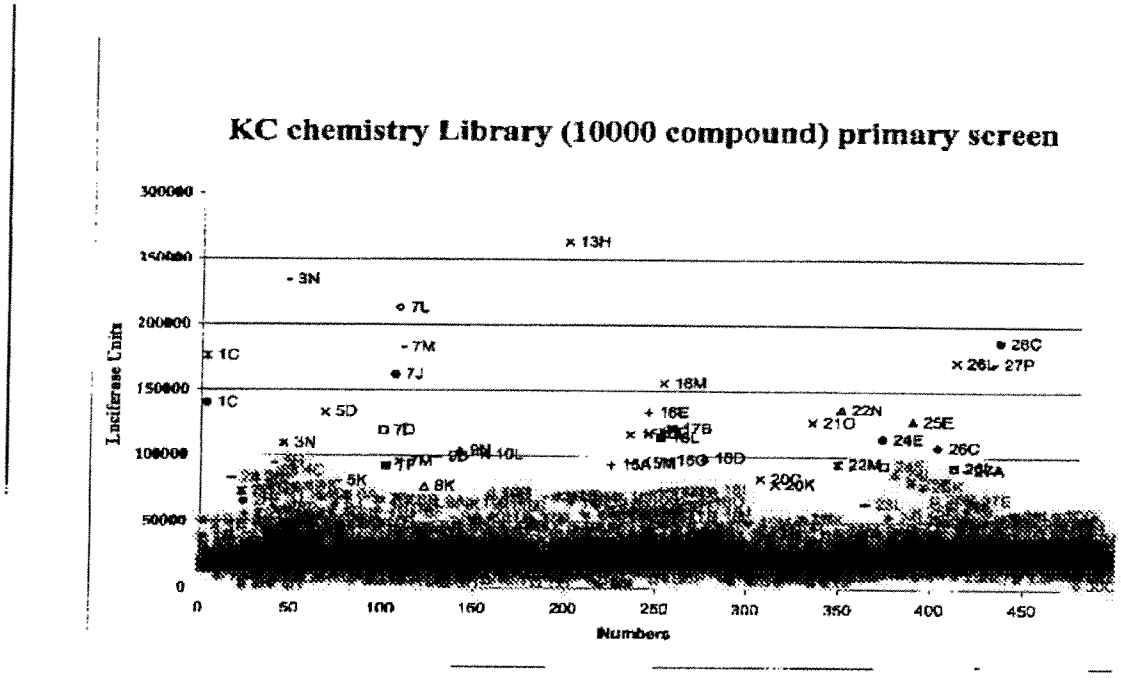


Figure 2. Results of primary screen of 10 000 membered library against FXR

The 25 most active compounds at 10 μM were re-synthesized to confirm their structure and activity. In collaboration with Dr K.C. Nicolaou's group (Scripps Research Institute, La Jolla, CA) smaller "focused" chemical libraries were designed and prepared around these hits and subjected to multiple rounds of screening. The design and rational of smaller and more focused libraries around the initial hits identified from primary screen is represented in figure 3. Through this iterative process a total of seven additional rounds of synthesis and selection was conducted resulting in a novel compound that was as effective as a proprietary synthetic ligand developed by Glaxo-Smith-Kline (GW4064) in cell based assays. See figures 4a,b and c. Using this identified compound fexaramate (EC_{50} 127nM) as a scaffold three additional focused libraries were made and screened to obtain at least four classes of potent, non-steroidal FXR agonists termed fexarene (EC_{50} 36nM), fexaramine EC_{50} 36nM), fexarine (EC_{50} 25nM) and fexarchloramide (EC_{50} 188nM). EC_{50} values determined via the activity in the cell based assay previously described with Prism 3.0 software.

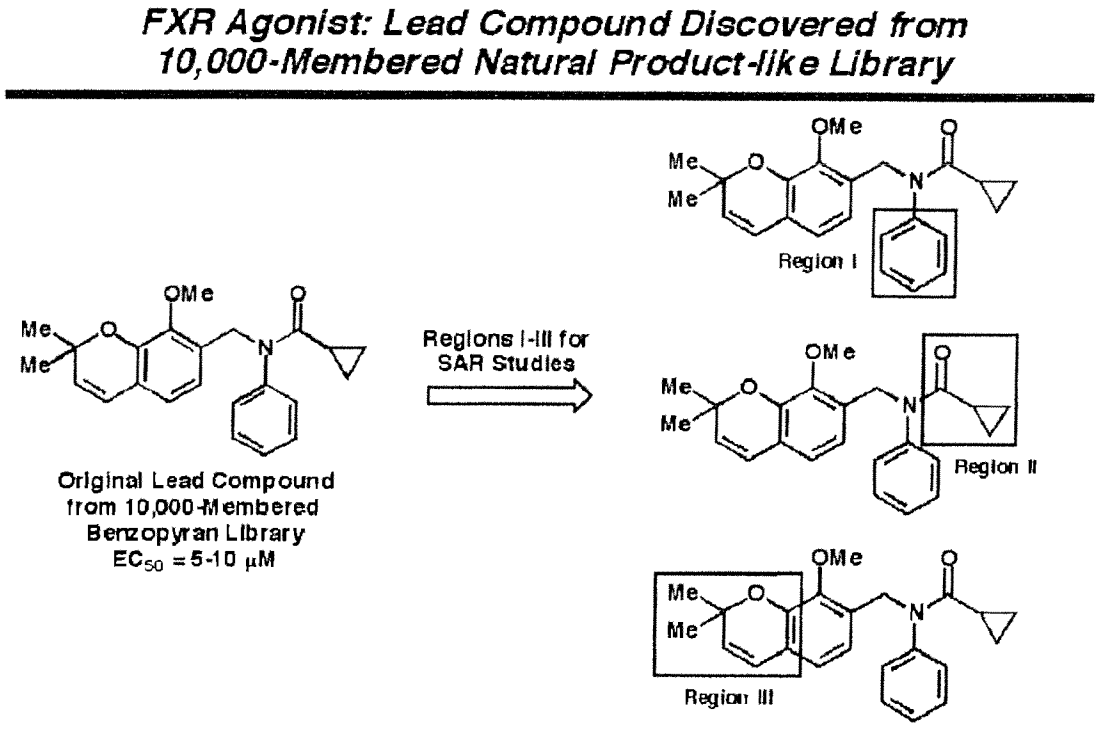


Figure 3: Design and Rational of smaller and more focused libraries around the initial hits identified from primary screen.

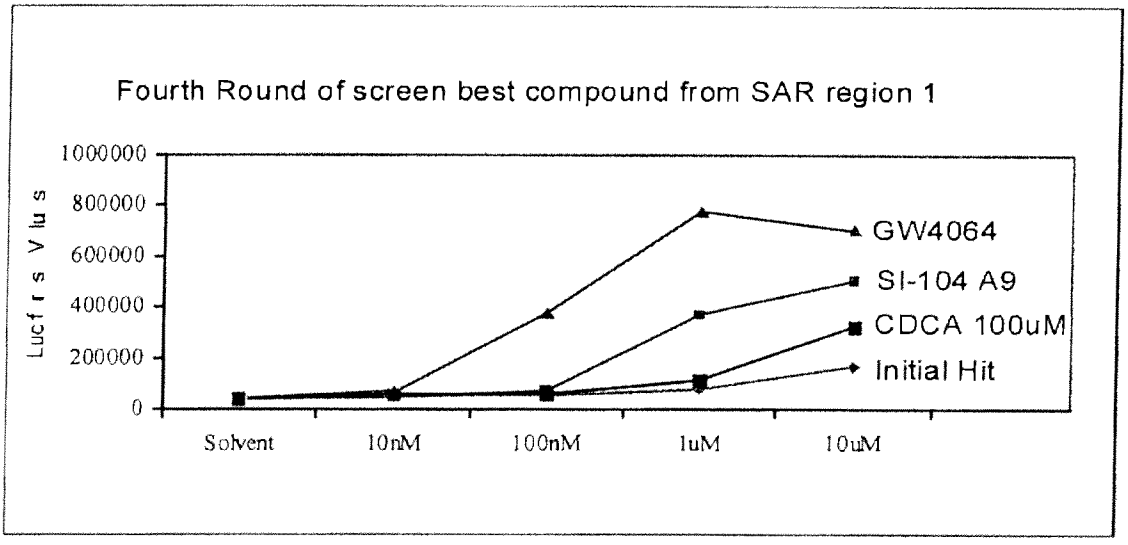


Figure 4a: Best compound identified from focused libraries designed around SAR region 1.

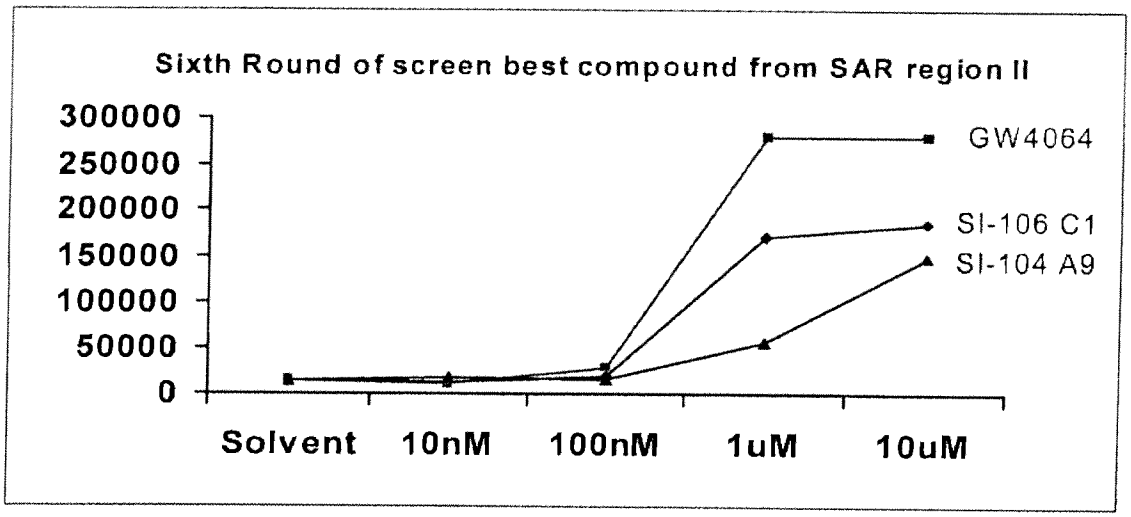


Figure 4b: Best compound identified from focused libraries designed around SAR region II.

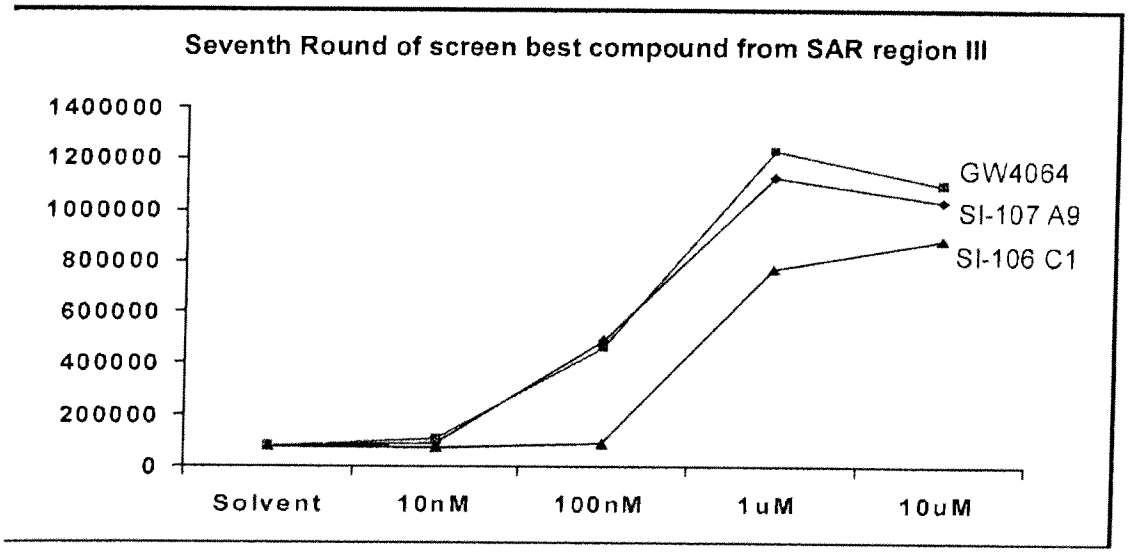


Figure 4c: Best compound identified from focused libraries designed around SAR region III.